ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1991, p. 1626–1634
0066-4804/91/081626-09$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 35, No. 8

Bacteremia Caused by Hemolytic, High-Level Gentamicin-Resistant *Enterococcus faecalis*

MARK M. HUYCKE,[1]* CAROL A. SPIEGEL,[2]† AND MICHAEL S. GILMORE[3]‡

Departments of Medicine[1] and Pathology and Laboratory Medicine,[2] University of Wisconsin, Madison, Wisconsin 53706, and Departments of Medicine[1] and Microbiology and Immunology,[3] University of Oklahoma, Oklahoma City, Oklahoma 73190

Received 9 November 1990/Accepted 3 June 1991

Between 1 January 1984 and 31 December 1987, 206 enterococcal blood isolates at the University of Wisconsin Hospital and Clinics were analyzed for high-level aminoglycoside resistance (hereafter high-level aminoglycoside resistance is simply referred to as "resistance") and hemolysin production. Of 190 *Enterococcus faecalis* isolates, 68 (35.8%) were resistant to gentamicin. Of these 68 strains, 67 (98.5%) contained a gene coding for the bifunctional aminoglycoside-modifying 6′-aminoglycoside acetyltransferase-2″-aminoglycoside phosphotransferase [AAC(6′)-APH(2″)] enzyme. Of 190 isolates, 85 (44.7%) were hemolytic and contained a gene coding for component A of the enterococcal hemolysin. Sixty-two of 68 (91.2%) gentamicin-resistant isolates but only 23 of 122 (18.8%) gentamicin-susceptible isolates were hemolytic ($P < 0.001$). Twelve of the hemolytic, gentamicin-resistant *E. faecalis* blood isolates, but only 2 of 9 nonhemolytic or gentamicin-susceptible isolates, had identical chromosomal DNA restriction endonuclease digestion patterns, suggesting a common derivation for these strains. A historical cohort study from 1 July 1985 to 31 March 1987 identified by regression analysis postsurgical intensive care unit status (odds ratio [OR], 5.0; 95% confidence interval [CI], 1.1 to 22.8) and prior treatment with an expanded- or broad-spectrum cephalosporin (OR, 3.0; 95% CI, 0.9 to 10.1) as risk factors for gentamicin-resistant *E. faecalis* bacteremia. Patients with hemolytic, gentamicin-resistant *E. faecalis* bacteremia had a fivefold-increased risk for death within 3 weeks of their bacteremia compared with patients with nonhemolytic, gentamicin-susceptible strains (95% CI, 1.0 to 25.4).

Enterococci are the third leading cause of nosocomial infection and the sixth leading cause of hospital-acquired bacteremia in the United States (9). Numerous studies report 30 to 68% case/fatality ratios for patients with enterococcal bacteremia (3, 7, 13, 16, 21, 32, 35–37, 40, 50, 54, 66). Although preexisting debilitating conditions and concomitant infections contributed to high overall mortality rates in these studies, death was attributed directly to enterococcal sepsis in 7 to 50% of fatal cases (7, 35, 37, 50, 54, 64). Similarly, an analysis of 500 bloodstream infections at the University of Colorado Hospital identified enterococci as the only gram-positive organism independently associated with a high risk of death (63). Yet, despite the poor prognosis associated with nosocomial enterococcal bacteremia, little is known of microbial determinants that contribute to adverse outcomes.

Antibiotic resistance (22, 45) or hemolysin production (27) by enterococci might interfere with adequate treatment of deep-seated enterococcal infections. Enterococcal infection caused by strains with high-level resistance to gentamicin (MIC > 2 mg/ml) (hereafter high-level aminoglycoside resistance is simply referred to as "resistance") has been reported (2, 22, 48, 60, 68, 69). Factors predisposing patients to the acquisition of resistant enterococci include hospitalization longer than 2 weeks (2), receipt of multiple antibiotics (2) including cephalosporins or aminoglycosides (68), and the number of surgical procedures (68). However, in these studies, few patients had bacteremia (2, 48, 68, 69) or clinical outcome was not analyzed (60).

Some enterococcal strains produce a hemolysin (14) which has general membrane lytic properties affecting both eukaryotic cells (hemolysin) and prokaryotic cells (bacteriocin) (4, 29). The hemolysin is secreted in an inactive form termed component L (20), which is cleaved by a second factor, component A (19), to form the active lytic factor. Human and horse erythrocytes are susceptible to the lytic action of active component L, while sheep erythrocytes are resistant (4). In a murine model of peritoneal infection, the 50% lethal dose (LD_{50}) for a nonhemolytic mutant of *Enterococcus faecalis* was 10-fold higher than the LD_{50} for the hemolytic wild-type strain (26). One group of Japanese investigators reported that 60% of *E. faecalis* isolates from human infections were hemolytic compared with 14% of fecal isolates from healthy individuals (27).

In order to further study enterococcal bloodstream infections, we have determined the frequency of bacteremia due to gentamicin-resistant *E. faecalis*, the frequency of hemolysin production in these strains, and the correlation of these and other parameters with clinical outcome in a large group of patients at the University of Wisconsin Hospital and Clinics.

MATERIALS AND METHODS

Study periods. This investigation encompasses three overlapping study periods. From the Clinical Microbiology Laboratory records, we identified the number of enterococcal blood isolates between 1 January 1982 and 31 December 1987. Only those strains obtained after 1 January 1984 were available for further testing. A cohort study was performed

* Address for correspondence: Division of Infectious Diseases, Department of Veterans Affairs Medical Center, 921 N.E. 13th St., Oklahoma City, OK 73104.

† Present address: University of Wisconsin Hospital and Clinics, Madison, WI 53792-0001.

‡ Present address: University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

on patients who had enterococcal bacteremia from July 1985 through March 1987.

Microbiologic methods. (i) Source of strains. All premortem enterococcal blood isolates from January 1984 through December 1987 were identified from records of the Clinical Microbiology Laboratory. Multiple isolates from a patient were enumerated separately when the cultures were obtained ≥10 days apart, when aminoglycoside susceptibility test results differed (see below), or when the strains were of different species. Blood culture isolates were stored in brain heart infusion broth with 10% glycerol at −70°C. Isolates were revived from frozen stock by subculture onto Trypticase soy agar with 5% sheep blood (BAP; BBL, Cockeysville, Md.) and incubated at 35°C in ambient air overnight. Multiple isolates from the same patient were tested only if the blood cultures had been collected more than 2 days apart. A commercially available kit was used to identify the strains to species level (API 20S; Analytab Products, Inc., Plainview, N.Y.).

(ii) Susceptibility testing. Susceptibility testing to gentamicin sulfate, kanamycin monosulfate, tobramycin (free base), streptomycin sulfate, amikacin (free base), netilmicin sulfate, neomycin sulfate, and spectinomycin dihydrochloride was performed by using macrobroth and microtiter methods as previously described (56, 71). Strains that grew in the presence of 2 mg of an aminoglycoside per ml were considered to possess resistance. Discrepancies between test methods were resolved by repeat testing or, if necessary, by time-kill studies as previously described (57).

(iii) β-Lactamase and hemolysin production. β-Lactamase activity was determined by using the nitrocefin (Glaxo Research Limited, Greenford, Middlesex, England) slide test (51). Hemolytic activity was determined after incubation for 2 days on blood agar base with 5% human blood.

(iv) DNA probes for aminoglycoside-modifying enzymes and hemolysin/bacteriocin. Radiolabeled probes were prepared by the incorporation of α-^{32}P-labeled-deoxyribonucleotides, using a random primed DNA labeling kit in accordance with the manufacturer's instructions (U.S. Biochemical Corporation, Cleveland, Ohio). DNA probes were employed in whole-colony blot hybridization under conditions of high stringency to determine the presence of homologous genomic DNA (39).

Aminoglycoside resistance in enterococci has previously been associated with a gene encoding an enzyme which has both 2″-phosphorylating and 6′-acetylating activity (61). Substrates for this 6′-aminoglycoside acetyltransferase-2″-aminoglycoside phosphotransferase [AAC(6′)-APH(2″)] enzyme include gentamicin, kanamycin, amikacin, tobramycin, and netilmicin (8, 12). Two DNA probes were developed for this enzyme. A 781-bp *Alu*I-*Sca*I fragment was derived from a recombinant plasmid, pSF815AC (kindly donated by J. J. Ferretti), containing a portion of the gene encoding 6′-acetylating activity of AAC(6′)-APH(2″) which originated in *E. faecalis* (15). A second 767-bp *Sca*I-*Alu*I fragment was derived from a recombinant plasmid pSF815AP (kindly donated by J. J. Ferretti), which included the portion of the structural gene encoding 2″-phosphorylating activity of AAC(6′)-APH(2″) (15).

To identify hemolytic potential, we developed a probe for component A, the factor necessary for activation of component L. A 1,245-bp DNA fragment specifying the *E. faecalis* pAD1-encoded hemolysin component A gene on recombinant plasmid pRAS18-3E (25) was generated by the polymerase chain reaction (44), using custom oligonucleotides to synthesize DNA from the initiator to the terminator codon (53).

(v) Isolation of plasmid DNA. *E. faecalis* cells grown overnight in sheep blood tryptic soy agar (Baxter Scientific Products, McGaw Park, Ill.) supplemented with glycine were lysed by using mutanolysin at 5×10^4 U/liter and lysozyme at 10 g/liter. Plasmid DNA was isolated for agarose gel electrophoresis as previously described (6).

(vi) Restriction fragment analysis of chromosomal DNA. Genomic DNA from enterococcal blood isolates was prepared as described by Murray et al. (46). Agarose plugs containing lysed cells were digested with *Sma*I (Bethesda Research Laboratories, Inc., Gaithersburg, Md.), electrophoresed using a contour-clamped homogeneous electric field device (CHEF-DRII; Bio-Rad, Richmond, Calif.) with the pulse time ramped from 5 to 35 s over 24 h at 200 V. Gels were stained with ethidium bromide and photographed with UV illumination.

Setting. The University of Wisconsin Hospital and Clinics is a 536-bed tertiary care institution. In addition to medical-surgical, pediatric, psychiatric, and eating disorders wards, there are five intensive care units including (i) a 12-bed trauma and life support center with four isolation rooms, (ii) a 7-bed medical intensive care unit with five isolation rooms, (iii) a 9-bed surgical intensive care unit with two isolation rooms, (iv) a 9-bed pediatric intensive care unit with four isolation rooms, and (v) a four-room burn unit with 7 isolation beds. The trauma and life support center and burn units are in adjacent locations on one ward, while other units are located throughout the hospital. The burn unit routinely keeps patients in strict isolation, while other units maintain patient isolation only as necessary per infection control protocols. For this reason, burn unit patients were considered separately from other patients. Patient transfers between nonpediatric intensive care units were common (decision to transfer based both on illness and bed availability). House staff and other staff regularly rotated through and worked in several of the intensive care units.

Study design. (i) Patient selection. The first recognized case of gentamicin-resistant *E. faecalis* bacteremia occurred in July 1985. A cohort of patients with *E. faecalis* bacteremia was constructed by reviewing all charts on patients with premortem *E. faecalis* blood isolates from July 1985 through March 1987. *E. faecalis* blood isolates were considered to represent true bacteremia if (i) two or more blood cultures yielded *E. faecalis* or (ii) when only one blood culture was positive, the clinical condition was consistent with bacteremic infection, or a local site of enterococcal infection had been confirmed by culture, or both.

During the cohort study period, the Clinical Microbiology Laboratory cultured 102 premortem *E. faecalis* blood isolates from 80 patients. Charts on these patients were reviewed. For patients with more than one admission during which an *E. faecalis* bacteremia had occurred, only the first admission was analyzed. Two patients were excluded because they had a single *E. faecalis* blood isolate in the absence of documented local enterococcal infection or clinical illness suggestive of bacteremia. Two enterococcal isolates were not tested for aminoglycoside susceptibility because one could not be revived from frozen stock and the other had not been stocked. As a result, one additional patient was excluded from the study. The cohort consisted of 77 patients with 98 episodes of enterococcal bacteremia: 36 patients with an initial *E. faecalis* bacteremia due to a gentamicin-resistant strain and 41 patients with an initial *E. faecalis* bacteremia due to a gentamicin-susceptible strain.

Final *E. faecalis* blood isolates for two patients with multiple bacteremias were not available for testing, so for outcome analyses the cohort consisted of 75 patients.

(ii) Study definitions. Clinical and epidemiologic data were obtained by chart and laboratory culture record review. Data collected for each patient included demographic information, underlying illness, unit or ward location at the time of the initial *E. faecalis* blood isolation (or prior unit or ward if transferred within the preceding 72 h), number of operations, and length of hospitalization. *E. faecalis* blood isolates obtained 72 or more hours after admission without laboratory evidence of enterococcal infection within that time period were defined as hospital acquired; all other blood isolates were considered community acquired (65). Persistent or recurrent *E. faecalis* bacteremia was defined as having occurred when additional blood cultures were collected 10 days to 12 weeks after the initial episode. Associated enterococcal infections were defined according to established criteria (65). Enterococcal isolates from sources other than blood were not routinely saved and therefore were unavailable for antimicrobial susceptibility testing.

Parenteral antimicrobial therapy, exclusive of perioperative prophylaxis, given between admission and the initial *E. faecalis* blood isolate was noted for each patient and categorized as follows: (i) cell wall synthesis inhibitors able to limit growth of most enterococci at achievable drug levels in serum (e.g., penicillin, ureido-penicillins, vancomycin, imipenem, narrow-spectrum cephalosporins), (ii) expanded- and broad-spectrum cephalosporins, and (iii) aminoglycosides. While narrow-spectrum cephalosporins are not appropriate agents for treating enterococcal infections, they have inhibitory activity against many enterococcal isolates at achievable concentrations in serum and are less often associated with gram-positive superinfections compared with many expanded- and broad-spectrum cephalosporins (31, 59, 62). For this reason, expanded- and broad-spectrum cephalosporins were analyzed separately. Antimicrobial therapy for *E. faecalis* bacteremia was considered appropriate if single or combined agents with proven efficacy in the treatment of enterococcal infection were given parenterally for 7 days or more (36). Therapy was considered inadequate if antimicrobial agents were not given, if antimicrobial agents were given orally or for less than 7 days regardless of efficacy, or if the antimicrobial agents given lacked in vitro activity against the patient's strain.

Statistics. Continuous (Student's *t* test) and categorical (Fisher's exact test and chi-square test) variables for the study and control groups were compared ($\alpha = 0.05$; two tail). The standard deviation for the distribution of single observations is reported as indicated. Variables identified by univariate analysis as somewhat ($0.05 < P < 0.20$) or significantly ($P < 0.05$) associated with gentamicin-resistant *E. faecalis* bacteremia or outcome were determined in the presence of other variables by logistic regression and expressed as an odds ratio (OR) with confidence intervals (CI) (33, 52).

RESULTS

Aminoglycoside resistance patterns for *E. faecalis* blood isolates. There were 284 enterococcal blood isolates during the study period (January 1982 to December 1987) including the 102 premortem *E. faecalis* isolates from the patients in the cohort study (July 1985 to March 1987). Seventy-eight strains could not be tested because they were no longer available (77 strains) or were nonviable (1 strain). Of the remaining 206 strains, 190 (92.2%) were *E. faecalis*, 15 (7.3%) were *Enterococcus faecium*, and one (0.5%) was *Enterococcus avium*. Figure 1 shows the semiannual occurrence of enterococcal blood isolates from 1984 through 1987. The initially recognized blood isolates with resistance to streptomycin, gentamicin, and both streptomycin and gentamicin occurred in January 1984, July 1985, and September 1985, respectively. Despite semiannual increases in enterococcal blood isolates in 1986 and 1987 compared with the preceding 4 years, no statistically significant increase in their occurrence was found (data not shown).

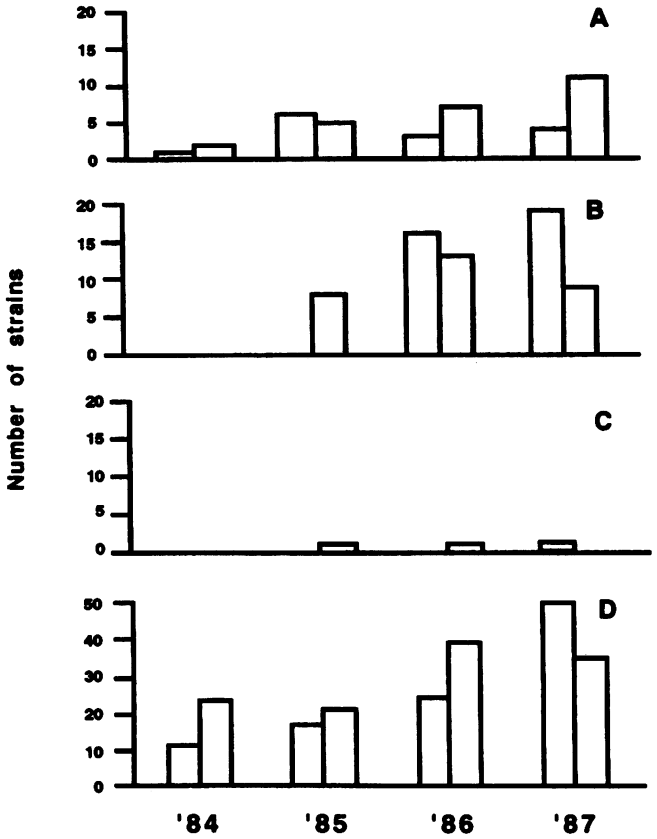

FIG. 1. Semiannual occurrence of enterococcal blood isolates with resistance to streptomycin (A), gentamicin (B), and both streptomycin and gentamicin (C) at the University of Wisconsin Hospital and Clinics from January 1984 to December 1987. Graph D shows the total number of blood isolates for each period. Thirteen, one, and one isolate were unavailable for susceptibility testing in 1984, 1985, and 1986, respectively.

Strains could be placed into 11 groups (I to XI [numbered chronologically by date of first isolation]) on the basis of their phenotypic resistance pattern to eight aminoglycosides (Table 1). It is of note that all gentamicin-resistant strains were kanamycin resistant. The 112 strains resistant to any agent in the aminoglycoside battery were tested for β-lactamase activity, and all were negative.

DNA hybridization studies for *E. faecalis* blood isolates. Of 68 gentamicin-resistant isolates (groups V, VI, VIII, and X in Table 1), 67 (98.5%) were positive for APH(2″) and AAC(6′) portions of the AAC(6′)-APH(2″) gene; of 122 gentamicin-susceptible strains, 2 (1.6%) had evidence of one or both genes ($P < 0.001$ by chi-square test). Of 190 *E. faecalis* isolates, 85 (44.7%) were hemolytic on human blood

TABLE 1. Aminoglycoside resistance and hemolysin characteristics for 190 *E. faecalis* blood isolates

Group	Date (mo/yr) of first isolate[a]	Aminoglycoside resistance phenotype[b]								No. of strains with gene[c]:			No. of strains
		Gm	Ak	Tm	St	Km	Nt	Ne	Sp	AAC(6')	APH(2")	Hemolysin[d]	
I	1/84									1	0	10	82
II	6/84				R	R		R		1	1	8	31
III	8/84				R					0	0	2	3
IV	11/84				R	R		R	R	0	0	1	2
V	7/85	R		R		R		R		63	63	59	63
VI	9/85	R		R	R	R		R		2	2	1	2
VII	9/85				R			R		0	0	0	1
VIII	8/86	R			R	R		R		0	0	0	1
IX	3/87			R				R		0	0	2	2
X	7/87	R				R				2	2	2	2
XI	9/87				R				R	0	0	0	1
I–XI										69	68	85	190

[a] First isolate during the study period beginning January 1984.
[b] Results when tested by microtiter. R, resistant (MIC ≥ 2 mg/ml). All others were susceptible. Abbreviations: Gm, gentamicin; Ak, amikacin; Tm, tobramycin; St, streptomycin; Km, kanamycin; Nt, netilmicin; Ne, neomycin; Sp, spectinomycin. See text for methods.
[c] AAC(6'), DNA fragment from pSF815AC coding for aminoglycoside 6'-acetyltransferase portion of the AAC(6')-APH(2") enzyme; APH(2"), DNA fragment from pSF815AP coding for the aminoglycoside 2"-phosphotransferase activity; hemolysin, DNA fragment from pRAS18-3E coding for component A. See text for methods.
[d] All isolates positive for component A were hemolytic on human blood agar.

agar, and each isolate contained the hemolysin component A gene. None of the nonhemolytic *E. faecalis* isolates were positive for the component A gene. Thus, the presence of the component A gene accurately predicted hemolysin production phenotype. Sixty-two of 68 (91.2%) gentamicin-resistant isolates and 23 of 122 (18.8%) gentamicin-susceptible isolates contained the component A gene. The association of gentamicin resistance and hemolysin production was highly significant ($P < 0.001$ by chi-square test).

Historical cohort study. Characteristics of patients with *E. faecalis* bacteremia caused by gentamicin-resistant or gentamicin-susceptible strains were similar. There were no significant differences between these groups for the following factors: age, sex, length of hospitalization or number of operations before the first positive *E. faecalis* blood culture, duration of hospitalization, primary underlying illness, prior treatment with aminoglycosides or cell wall active agents able to limit growth of enterococci, enterococcal infection at another site, polymicrobial bacteremia, and recurrent or persistent enterococcal bacteremia (chi-square or Student's *t* test *P* values all >0.20; data not shown).

Variables tending toward or significantly associated with gentamicin-resistant *E. faecalis* bacteremia are shown in Table 2. Prior treatment with expanded- or broad-spectrum cephalosporins and hospitalization in a nonburn intensive care unit both appeared to be risk factors for gentamicin-resistant enterococcal bacteremia.

To further evaluate associations between gentamicin-re-

TABLE 2. Comparisons between selected risk factors and gentamicin activity for 77 patients with *E. faecalis* bacteremia

Baseline variable	No. of patients (%) with *E. faecalis* bacteremia		*P* value[a]
	Gentamicin-susceptible strain (n = 41)	Gentamicin-resistant strain (n = 36)	
Type of bacteremia			
Community-acquired	7 (17)	2 (6)	0.16[b]
Hospital-acquired	34 (83)	34 (94)	
Expanded- or broad-spectrum cephalosporin			
Received	11 (27)	18 (50)	0.04
Not received	30 (95)	18 (50)	
Nursing unit			
Intensive care	21 (51)	28 (78)	0.05
Burn	6 (15)	3 (8)	
Other	14 (34)	5 (14)	
Nursing unit/underlying illness			
Intensive care/trauma	7 (17)	8 (22)	0.13
Intensive care/surgery	6 (15)	12 (33)	
Intensive care/other	8 (20)	8 (22)	
Burn/burn	6 (15)	3 (8)	
Other/any illness	14 (34)	5 (14)	

[a] *P* value for the group indicated by the Chi-square test unless otherwise specified.
[b] Fisher's exact test.

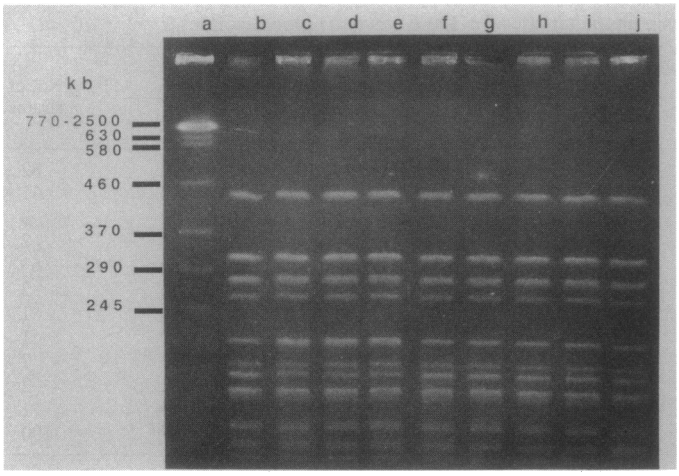

FIG. 2. Chromosomal digestion patterns for gentamicin-resistant *E. faecalis* blood isolates from the trauma and life support center between July 1985 and December 1985 (lanes b to j); all strains were hemolytic. Lane a contains a chromosomal DNA digest from *Saccharomyces cerevisiae* YNN295 (Bio-Rad).

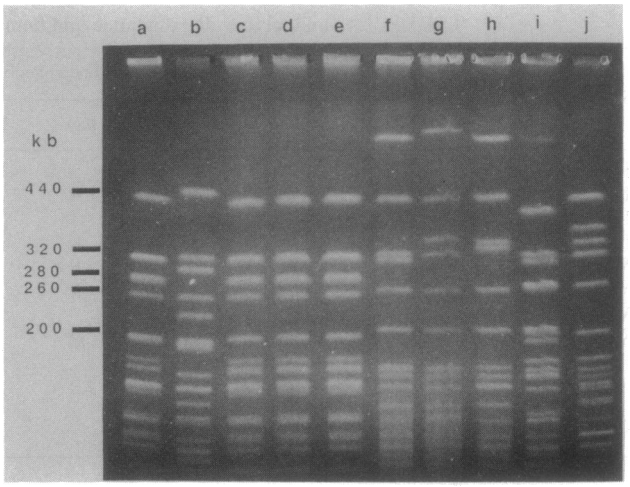

FIG. 3. Chromosomal digestion patterns for hemolytic, gentamicin-resistant *E. faecalis* blood isolates from 1986 (lanes c to e) and hemolytic, gentamicin-susceptible *E. faecalis* blood isolates (lanes f to j). Lane b contains chromosomal DNA from a community-acquired, nonhemolytic, gentamicin-resistant *E. faecalis* blood isolate. Lane a contains chromosomal DNA from the hemolytic, gentamicin-resistant *E. faecalis* strain shown in lane c of Fig. 2.

sistant *E. faecalis* bacteremia and hospitalization in nonburn intensive care units, daily location was analyzed for the 15 patients identified during the first 6 months of the outbreak from July through December 1985. Seven patients were bacteremic with gentamicin-resistant strains, and all had been or were still in the trauma and life support center prior to their initial *E. faecalis* bacteremia. The seven patients with gentamicin-resistant strains spent more days per patient in the trauma and life support center (28.0 ± 17.2 [mean ± standard deviation]) than did the eight patients with gentamicin-susceptible strains (3.5 ± 3.1; $P = 0.005$ by Student's t test).

Relatedness of selected *E. faecalis* blood isolates was assessed by analyzing plasmid and chromosomal DNA. Eight gentamicin-resistant strains recovered from patients in the trauma and life support center in 1985 contained one to five plasmids. The six plasmid profile patterns found suggested that these strains were not the same or of a recent derivative. However, the chromosomal DNA restriction endonuclease digestion patterns for 27 selected *E. faecalis* blood isolates (Fig. 2 to 4) revealed a common pattern for hemolytic, gentamicin-resistant strains. Twelve hemolytic, gentamicin-resistant strains obtained from 10 patients over 17 months had identical patterns (Fig. 2, lanes b to j; Fig. 3, lanes c to e). None of 9 nonhemolytic, gentamicin-susceptible strains had patterns that matched the pattern of hemolytic, gentamicin-resistant strains (Fig. 4, lanes b to j). The two nonhemolytic, gentamicin-susceptible strains with a matching pattern (Fig. 4, lanes d and j) were obtained from one patient (who was in the trauma and life support center) at an interval of 7 weeks. A community-acquired, nonhemolytic, gentamicin-resistant *E. faecalis* isolate (Fig. 3, lane b) had a pattern that differed from that of the hemolytic, gentamicin-resistant isolates. Five hemolytic, gentamicin-susceptible strains (Fig. 3, lanes f to j) from 1984 ($n = 3$), 1985 ($n = 1$), and 1987 ($n = 1$) had patterns that differed from each other and the pattern of the hemolytic, gentamicin-resistant isolates.

A majority of patients in the study were severely ill; 75% were cared for in an intensive care unit at the time of their initial *E. faecalis* bacteremia. Variables somewhat or strongly associated with the development of gentamicin-resistant *E. faecalis* bacteremia, as shown in Table 2, were used to develop a multiple logistic regression model. Results in Table 3 quantify the risk of gentamicin-resistant bacteremia independent of confounding variables. Patients recovering from recent major surgery in a nonburn intensive care unit were five times more likely to have *E. faecalis* bacteremia caused by a gentamicin-resistant strain than by a gentamicin-susceptible strain. Patients receiving expanded- or broad-spectrum cephalosporins had a threefold-increased risk for bacteremia with a gentamicin-resistant strain.

Mortality in the cohort was 31% (24 of 77). Of the 24 fatalities, 14 (58%) occurred within 3 weeks following an *E. faecalis* bacteremia; deaths in the remaining 10 patients

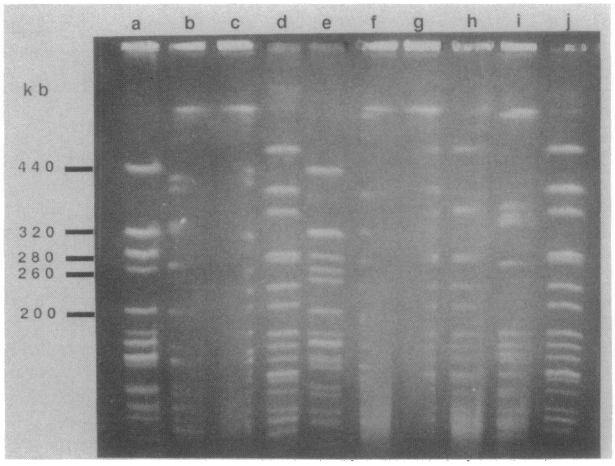

FIG. 4. Chromosomal digestion patterns for gentamicin-susceptible *E. faecalis* blood isolates from the trauma and life support center (lanes b and e to j) and other units (lanes c and d) between July 1985 and October 1985. Lane a contains chromosomal DNA from the hemolytic, gentamicin-resistant *E. faecalis* strain shown in lane h of Fig. 2.

TABLE 3. Logistic regression analysis of risk factors associated with gentamicin-resistant *E. faecalis* bacteremia

Baseline variable	*P* value	OR[a]	95% CI
Hospital-acquired bacteremia	0.27	2.9	0.4–19.2
Nursing unit/underlying illness			
Intensive care/trauma	0.12	3.6	0.7–17.5
Intensive care/surgery	0.04	5.0	1.1–22.8
Intensive care/other	0.25	2.5	0.5–11.3
Burn/burn	0.88	1.1	0.2–6.8
Prior antimicrobial therapy			
Selected cell wall synthesis inhibitors	0.20	0.3	0.05–1.9
Expanded- or broad-spectrum cephalosporins	0.08	3.0	0.9–10.1
Aminoglycosides	0.51	1.7	0.4–7.8

[a] The reference category is 1.0.

occurred at widely different intervals more than 4 weeks after an enterococcal bacteremia. Therefore, patients dying within 3 weeks of an *E. faecalis* bacteremia were defined as having an acutely terminal outcome. Univariate analyses detected no significant differences between patient groups with acutely terminal or other outcomes for the following factors: sex; type of bacteremia; secondary underlying illness; burn as a primary illness; prior treatment with antienterococcal antibiotics, aminoglycosides, or expanded- or broad-spectrum cephalosporins; associated focal enterococcal infection; ward location; polymicrobial bacteremia; and antibiotic efficacy for the initial *E. faecalis* bacteremia (chi-square *P* values all >0.20; data not shown).

Table 4 lists univariate analyses of variables somewhat or strongly associated with an acutely terminal outcome. These variables were analyzed by logistic regression, and results of the model are shown in Table 5. Both hemolysin production phenotype for the infecting *E. faecalis* strain and patient age were independently associated with an acutely terminal outcome, i.e., independent of duration of hospitalization or underlying illness categorized by unit location. A similar OR value was obtained when the gentamicin resistance phenotype was substituted for the hemolysin phenotype. This was expected because virtually all gentamicin-resistant strains were hemolytic. Patients with acutely terminal outcomes were hospitalized an average of 2 weeks before their initial *E. faecalis* bacteremia, compared with 6 weeks for patients with other outcomes. Accordingly, risk associated with length of hospitalization was stratified to greater and less than 3 weeks. Shorter hospitalization, however, was only marginally associated with an acutely terminal outcome.

TABLE 4. Comparison between selected prognostic indicators and outcome for 75 patients with *E. faecalis* bacteremia

Baseline variable	Value for group: Acutely terminal outcome (*n* = 14)	Value for group: Other outcome (*n* = 61)	*P* value[a]
Age (yr [mean ± SD])	60 ± 15	47 ± 24	0.01[b]
Length of hospitalization prior to first bacteremia (days [mean ± SD])	14 ± 13	37 ± 60	0.009[b]
Strain phenotype (no. of patients [%])			
Hemolytic	10 (71)	29 (48)	0.11
Nonhemolytic	4 (29)	32 (52)	
Primary underlying illness (no. of patients [%])			
Multiple trauma	4 (29)	11 (18)	0.09
Burn	0 (0)	9 (15)	
Recent surgery	2 (14)	21 (36)	
Other	8 (57)	19 (31)	
Nursing unit/underlying illness (no. of patients [%])			
Intensive care/trauma	3 (21)	11 (18)	0.13
Intensive care/surgery	1 (7)	17 (28)	
Intensive care/other	5 (36)	10 (16)	
Burn/burn	0 (0)	9 (15)	
Other/any illness	5 (36)	13 (23)	

[a] *P* value for the group indicated by the chi-square test unless otherwise specified.
[b] Student's *t* test.

We have described the appearance of gentamicin-resistant enterococcal bacteremias in a large trauma and life support unit. An unusually high percentage (91.2%) of the gentamicin-resistant *E. faecalis* blood isolates were hemolytic. During the 21-month study period, these hemolytic, gentamicin-

TABLE 5. Logistic regression analysis of 75 patients with *E. faecalis* bacteremia-defining predictors of an acutely terminal outcome

Baseline variable	*P* value	OR[a]	95% CI
Bacteremia caused by hemolytic *E. faecalis* strain[b]	0.05	5.0	1.0–25.4
Nursing unit/underlying illness			
Intensive care/trauma	0.41	0.4	0.06–3.1
Intensive care/surgery	0.07	0.1	0.01–1.2
Intensive care/other	0.61	1.6	0.3–8.4
Burn/burn[c]	0.28		
Age[d]	0.04	1.04	1.00–1.08
Hospitalization of <21 days before initial bacteremia	0.07	4.4	0.9–22.4

[a] The reference category is 1.0.
[b] Regression analysis substituting gentamicin resistance for the hemolysin phenotype resulted in OR = 5.0 (95% CI = 1.0 to 24.1).
[c] Relative risk could not be calculated because no burn patient experienced an acutely terminal outcome. The *P* value was determined by comparing the goodness-of-fit statistics for the full and reduced models.
[d] Relative risk predicts increased risk per year of age.

DISCUSSION

Severe enterococcal infections are best treated with a combination of two agents: an antibiotic able to limit growth of enterococci (e.g., penicillin or vancomycin) and an aminoglycoside to which the enterococcal strain lacks resistance (22, 24, 67). Enterococcal isolates resistant to streptomycin and kanamycin have long been recognized (38, 43). However, it was not until 1979 that the first *E. faecalis* strain resistant to gentamicin was reported (23). Since then, gentamicin-resistant strains have been identified worldwide (10, 11, 28, 41, 47, 48, 60, 61, 68) and shown to contain conjugative resistance plasmids that produce aminoglycoside-modifying enzyme(s) (10, 11, 23, 41, 70).

resistant strains made up 45% of the initial bacteremic isolates. Although variable plasmid profiles for the first eight hemolytic, gentamicin-resistant *E. faecalis* strains suggested several different strains, a single chromosomal DNA restriction endonuclease digestion pattern was found for these isolates. Gentamicin-susceptible isolates, irrespective of hemolysin production phenotype, demonstrated considerable restriction fragment length polymorphism. Quite likely a single hemolytic, gentamicin-resistant *E. faecalis* strain caused multiple bacteremias in the trauma and life support center before becoming a source of bacteremia in other intensive care units and wards 6 months later.

The reservoir and mode of transmission for hemolytic, gentamicin-resistant enterococci were not determined in this study. Our findings are consistent with the epidemiology of nosocomially acquired enterococcal infection described by Zervos and associates (68, 69). These researchers used plasmid content as an epidemiologic marker of strain identity and showed transmission of gentamicin-resistant enterococci between patients in intensive care units, probably through transient carriage on the hands of hospital personnel. The reservoir for these strains was not defined, but high rates of colonization for patients on the medical wards and residents of an adjacent nursing home were reported (72).

We have used DNA hybridization probes to demonstrate that almost all (67 of 68) gentamicin-resistant enterococci in our study carried the AAC(6')-APH(2") gene, which encodes an enzyme that can potentially inactivate all clinically relevant aminoglycosides except streptomycin (8, 12). None of the 190 isolates tested was resistant to 2 mg of amikacin or netilmicin per ml (Table 1). However, resistance to kanamycin, which was characteristic of our gentamicin-resistant isolates, is a reliable predictor of amikacin resistance (22). The substrate activity of AAC(6')-APH(2") would predict gentamicin-resistant strains to be indifferent to the killing effects of other clinically useful aminoglycosides (except streptomycin) when combined with cell wall active agents.

Patients treated with expanded- or broad-spectrum cephalosporins had a threefold-increased chance of having gentamicin-resistant organisms upon development of an enterococcal bacteremia. Some studies (3, 21, 43, 68, 69), but not all (7, 32, 37), have noted correlations between systemic antibiotic therapy, particularly cephalosporins, and nosocomial acquisition of gentamicin-resistant enterococci or occurrence of enterococcal infection. Since expanded- and broad-spectrum cephalosporins lack activity against enterococci, they predispose patients to enterococcal superinfection (42). This effect may be more dramatic in patients colonized by gentamicin-resistant strains.

The cohort mortality was 31% and is similar to the values found in other recent studies (3, 7, 13, 16, 21, 32, 35–37, 40, 50, 54, 66). Because determination of a primary cause of death for these critically ill patients was difficult, we stratified patients on the basis of length of time between the last positive blood isolate and death and used this as a measure of outcome. We found that fatality, for any reason, within 3 weeks of an *E. faecalis* bacteremia was independently associated with age and the isolation of a gentamicin-resistant, hemolytic *E. faecalis* strain. An increase in mortality with older age groups has generally been noted for nosocomial bacteremia (17, 34, 40, 55) and for enterococcal bacteremia in particular (7, 54). Although we, like others (21), were unable to show a correlation between appropriate antibiotic therapy for enterococcal bacteremia and clinical outcome, the proper application of antibiotics in patients with bloodstream infection should remain an important goal. Treatment guidelines for enterococcal infections can be found elsewhere (36).

An association between a gentamicin resistance, hemolysin production phenotype and adverse outcome for patients with enterococcal bacteremia has not been previously reported. The concept that hemolysins contribute to pathogenicity in other infections is well established. Streptococcal, pneumococcal, listerial, clostridial, and *Escherichia coli* hemolysins are recognized virulence factors (1, 5, 18, 30, 58). Hemolysins can inhibit leukocyte function (18, 49, 58) or provide iron for growing organisms in an iron-deficient milieu, such as human plasma and tissues (30). The enterococcal hemolysin was a virulence factor in both animal and in vitro models (26, 49). This study suggests it may play a similar role in human infection. However, our retrospective analysis of enterococcal bacteremia provides no absolute method for inferring the cause of a poor outcome in these patients despite attempts to control for underlying illness and patient location in a multivariate analysis (33). We cannot determine whether the hemolysin or aminoglycoside resistance phenotype, independently or in combination, led to an adverse outcome or whether these traits are markers for an as-yet-undefined causal factor. The association between the gentamicin resistance and hemolysin genes is not absolute; some strains have one or the other but not both. It will be important to determine their intracellular locations (plasmid versus bacterial chromosome) to better understand this phenomenon. However, their association suggests a distant clonal derivation for these strains in our hospital.

We agree with others that clinical laboratories should routinely identify enterococcal blood isolates to species level and screen them for resistance to gentamicin, kanamycin, and streptomycin (22). It should be emphasized that sheep, but not horse or rabbit, erythrocytes are insensitive to lysis by the enterococcal hemolysin (4). Because sheep erythrocytes are used commonly in screening for hemolysis by clinical microbiology laboratories, this phenotype can be easily overlooked. Whether hemolysin production by *E. faecalis* blood isolates should be routinely determined is unclear at this time.

ACKNOWLEDGMENTS

We are indebted to Debby Taniguchi and Susan Stolz for excellent technical support and to Jan Feyzi of the University of Wisconsin Biostatistics Center for statistical support.

Portions of this research were supported by Public Health Service grant EY 08289 and grants from the Presbyterian Health Foundation and the Oklahoma Center for the Advancement of Science and Technology (HR8-036).

REFERENCES

1. **Alouf, J. E., and M. Raynaud.** 1973. Purification and some properties of streptolysin O. Biochimie **55**:1187–1193.
2. **Axelrod, P., and G. H. Talbot.** 1989. Risk factors for acquisition of gentamicin-resistant enterococci. Arch. Intern. Med. **149**: 1397–1401.
3. **Barrall, D. T., R. K. Pardon, G. J. Slotman, and K. W. Burchard.** 1985. Enterococcal bacteremia in surgical patients. Arch. Surg. **120**:57–63.
4. **Basinger, S. F., and R. W. Jackson.** 1968. Bacteriocin (hemolysin) of *Streptococcus zymogenes*. J. Bacteriol. **96**:1895–1902.
5. **Berry, A. M., J. Yother, D. E. Briles, D. Hansman, and J. C. Paton.** 1989. Reduced virulence of a defined pneumolysin-negative mutant of *Streptococcus pneumoniae*. Infect. Immun. **57**:2037–2042.
6. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.

7. **Bryan, C. S., K. L. Reynolds, and J. J. Brown.** 1985. Mortality associated with enterococcal bacteremia. Surg. Gynecol. Obstet. **160**:557–561.
8. **Bryan, L. E.** 1984. Aminoglycoside resistance, p. 241–277. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., New York.
9. **Centers for Disease Control.** 1984. Infection surveillance [CDC Surveillance Summaries]. Morbid. Mortal. Weekly Rep. **35**: 17SS–29SS.
10. **Chen, H. Y., and J. D. Williams.** 1985. Transferable resistance and aminoglycoside-modifying enzymes in enterococci. J. Med. Microbiol. **20**:187–196.
11. **Courvalin, P., C. Carlier, and E. Collatz.** 1980. Plasmid-mediated resistance to aminocyclitol antibiotics in group D streptococci. J. Bacteriol. **143**:541–551.
12. **Davies, J. E.** 1986. Aminoglycoside-aminocyclitol antibiotics and their modifying enzymes, p. 790–809. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
13. **Dougherty, S. H., A. B. Flohr, and R. L. Simmons.** 1983. 'Breakthrough' enterococcal septicemia in surgical patients. Arch. Surg. **118**:232–237.
14. **Facklam, R. R.** 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. **23**:1131–1139.
15. **Ferretti, J. J., K. S. Gilmore, and P. Courvalin.** 1986. Nucleotide sequence analysis of the gene specifying the bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. J. Bacteriol. **167**:631–638.
16. **Garrison, R. N., D. E. Fry, S. Berberich, and H. C. Polk, Jr.** 1982. Enterococcal bacteremia: clinical implications and determinants of death. Ann. Surg. **196**:43–47.
17. **Gatell, J. M., A. Trilla, X. Latorre, M. Almela, J. Mensa, A. Moreno, J. M. Miro, J. A. Martinez, and J. G. San Miguel.** 1988. Nosocomial bacteremia in a large Spanish teaching hospital: analysis of factors influencing prognosis. Rev. Infect. Dis. **10**:203–210.
18. **Geoffroy, C., J.-L. Gaillard, J. E. Alouf, and P. Berche.** 1987. Purification, characterization, and toxicity of the sulfhydryl-activated hemolysin listeriolysin O from *Listeria monocytogenes*. Infect. Immun. **55**:1641–1646.
19. **Granato, P. A., and R. W. Jackson.** 1971. Characterization of the A component of *Streptococcus zymogenes* lysin. J. Bacteriol. **107**:551–556.
20. **Granato, P. A., and R. W. Jackson.** 1971. Purification and characterization of the L component of *Streptococcus zymogenes* lysin. J. Bacteriol. **108**:804–808.
21. **Gullberg, R. M., S. R. Homann, and J. P. Phair.** 1989. Enterococcal bacteremia: analysis of 75 episodes. Rev. Infect. Dis. **11**:74–85.
22. **Hoffmann, S. A., and R. C. Moellering, Jr.** 1987. The enterococcus: "putting the bug in our ears". Ann. Intern. Med. **106**:757–761.
23. **Horodniceanu, T., L. Bougueleret, N. El-Solh, G. Bieth, and F. Delbos.** 1979. High-level, plasmid-borne resistance to gentamicin in *Streptococcus faecalis* subsp. *zymogenes*. Antimicrob. Agents Chemother. **16**:686–689.
24. **Hunter, T. H.** 1947. Use of streptomycin in the treatment of bacterial endocarditis. Am. J. Med. **2**:436–442.
25. **Ike, Y., D. B. Clewell, R. A. Segarra, and M. S. Gilmore.** 1990. Genetic analysis of the pAD1 hemolysin/bacteriocin determinant in *Enterococcus faecalis*: Tn*917* insertional mutagenesis and cloning. J. Bacteriol. **172**:155–163.
26. **Ike, Y., H. Hashimoto, and D. B. Clewell.** 1984. Hemolysin of *Streptococcus faecalis* subspecies *zymogenes* contributes to virulence in mice. Infect. Immun. **45**:528–530.
27. **Ike, Y., H. Hashimoto, and D. B. Clewell.** 1987. High incidence of hemolysin production by *Enterococcus faecalis* strains associated with human parenteral infections. J. Clin. Microbiol. **24**:1524–1528.
28. **Ikeda, D. P., A. L. Barry, and S. G. Andersen.** 1984. Emergence of *Streptococcus faecalis* with high-level resistance to multiple aminocyclitol aminoglycosides. Diagn. Microbiol. Infect. Dis. **2**:171–177.
29. **Jackson, R. W.** 1971. Bacteriolysis and inhibition of gram-positive bacteria by components of *Streptococcus zymogenes* lysin. J. Bacteriol. **105**:156–159.
30. **Johnson, J. R., S. L. Moseley, P. L. Roberts, and W. E. Stamm.** 1988. Aerobactin and other virulence factor genes among strains of *Escherichia coli* causing urosepsis: association with patient characteristics. Infect. Immun. **56**:405–412.
31. **Jones, R. N.** 1985. Gram-positive superinfections following beta-lactam chemotherapy: the significance of the enterococcus. Infection **13**(Suppl. 1):S81–S88.
32. **Jones, W. G., P. S. Barie, R. W. Yurt, and C. W. Goodwin.** 1986. Enterococcal burn sepsis: a highly lethal complication in severely burned patients. Arch. Surg. **121**:649–652.
33. **Kleinbaum, D. G., L. L. Kupper, and H. Morgenstern.** 1982. Epidemiologic research: principles and quantitative methods. Lifetime Learning Publications, Belmont, Calif.
34. **Kreger, B. E., D. E. Craven, and W. R. McCabe.** 1980. Gram-negative bacteremia. IV. Re-evaluation of clinical features and treatment in 612 patients. Am. J. Med. **68**:344–355.
35. **Landry, S. L., D. L. Kaiser, and R. P. Wenzel.** 1989. Hospital stay and mortality attributed to nosocomial enterococcal bacteremia: a controlled study. Am. J. Infect. Control **17**:323–329.
36. **Maki, D. G., and W. A. Agger.** 1988. Enterococcal bacteremia: clinical features, the risk of endocarditis, and management. Medicine **67**:248–269.
37. **Malone, D. A., R. A. Wagner, J. P. Myers, and C. Watanakunakorn.** 1986. Enterococcal bacteremia in two large community teaching hospitals. Am. J. Med. **81**:601–606.
38. **Mandell, G. L., D. Kaye, M. E. Levison, and E. W. Hook.** 1970. Enterococcal endocarditis: an analysis of 38 patients observed at the New York-Cornell Medical Center. Arch. Intern. Med. **125**:258–264.
39. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **McGowan, J. E., M. W. Barnes, and M. Finland.** 1975. Bacteremia at Boston City Hospital: occurrence and mortality during 12 selected years (1935–1972), with special reference to hospital-acquired cases. J. Infect. Dis. **132**:316–335.
41. **Mederski-Samoraj, B. D., and B. E. Murray.** 1983. High-level resistance to gentamicin in clinical isolates of enterococci. J. Infect. Dis. **147**:751–757.
42. **Moellering, R. C., Jr.** 1982. Enterococcal infections in patients treated with moxalactam. Rev. Infect. Dis. **4**(Suppl.):S708–S711.
43. **Moellering, R. C., Jr., C. B. G. Wennersten, and A. N. Weinberg.** 1970. Prevalence of high-level resistance to aminoglycosides in clinical isolates of enterococci. Antimicrob. Agents Chemother. **10**:335–340.
44. **Mullis, K. B., and F. A. Faloona.** 1987. Specific synthesis of DNA in vitro via a polymerase chain reaction. Methods Enzymol. **155**:335–350.
45. **Murray, B. E., and B. Mederski-Samoraj.** 1983. Transferable β-lactamase: a new mechanism for in vitro penicillin resistance in *Streptococcus faecalis*. J. Clin. Invest. **72**:1168–1171.
46. **Murray, B. E., K. V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock.** 1990. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. **28**:2059–2063.
47. **Murray, B. E., J. Tsao, and J. A. Panida.** 1983. Enterococci from Bangkok, Thailand, with high-level resistance to currently available aminoglycosides. Antimicrob. Agents Chemother. **23**: 799–802.
48. **Nachamkin, I., P. Axelrod, G. H. Talbot, S. H. Fischer, C. B. Wennersten, R. C. Moellering, Jr., and R. R. MacGregor.** 1988. Multiply high-level-aminoglycoside-resistant enterococci isolated from patients in a university hospital. J. Clin. Microbiol. **26**:1287–1291.
49. **Novak, R. M., T. J. Holzer, and C. R. Liberin.** 1988. Human

neutrophil phagocytic killing of hemolysin-producing and nonhemolysin-producing *Enterococcus faecalis*. Clin. Res. **36**: 883A.

50. **Rimailho, A., E. Lampl, B. Riou, C. Richard, E. Rottman, and P. Auzepy.** 1988. Enterococcal bacteremia in a medical intensive care unit. Crit. Care Med. **16**:126–129.
51. **Rosenblatt, J. E.** 1986. Antimicrobial susceptibility testing of anaerobes, p. 129. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
52. **SAS Institute.** 1982. SAS user's guide: statistics—1982 edition. SAS Institute, Cary, N.C.
53. **Segarra, R. A., M. C. Booth, D. A. Morales, M. M. Huycke, and M. S. Gilmore.** 1991. Molecular characterization of the *Enterococcus faecalis* cytolysin activator. Infect. Immun. **59**:1239–1246.
54. **Shales, D. M., J. Levy, and E. Wolinsky.** 1981. Enterococcal bacteremia without endocarditis. Arch. Intern. Med. **141**:578–581.
55. **Spengler, R. F., W. B. Greenough, and P. D. Stolley.** 1978. A descriptive study of nosocomial bacteremias at The Johns Hopkins Hospital, 1968–1974. Johns Hopkins Med. J. **142**:77–84.
56. **Spiegel, C. A.** 1988. Laboratory detection of high-level aminoglycoside-aminocyclitol resistance in *Enterococcus* spp. J. Clin. Microbiol. **26**:2270–2274.
57. **Spiegel, C. A., and M. M. Huycke.** 1989. Endocarditis due to streptomycin susceptible *Enterococcus faecalis* with high-level gentamicin resistance. Arch. Intern. Med. **149**:1873–1875.
58. **Stevens, D. L., J. Mitten, and C. Henry.** 1987. Effects of alpha and theta toxins from *Clostridium perfringens* on human polymorphonuclear leukocytes. J. Infect. Dis. **156**:324–333.
59. **Thornsberry, C.** 1985. Review of in vitro activity of third-generation cephalosporins and other newer beta-lactam antibiotics against clinically important bacteria. Am. J. Med. **79**:14–20.
60. **Watanakunakorn, C.** 1989. The prevalence of high-level aminoglycoside resistance among enterococci isolated from blood cultures during 1980–1988. J. Antimicrob. Chemother. **24**:63–68.
61. **Weems, J. J., Jr., J. H. Lowrance, L. M. Baddour, and W. A. Simpson.** 1989. Molecular epidemiology of nosocomial, multiply aminoglycoside resistant *Enterococcus faecalis*. J. Antimicrob. Chemother. **24**:121–130.
62. **Weinstein, A. J., and A. L. Lentnek.** 1976. Cephalosporin-aminoglycoside synergism in experimental enterococcal endocarditis. Antimicrob. Agents Chemother. **9**:983–987.
63. **Weinstein, M. P., J. R. Murphy, L. B. Reller, and K. A. Lichtenstein.** 1983. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. II. Clinical observations, with special reference to factors influencing prognosis. Rev. Infect. Dis. **5**:54–69.
64. **Weinstein, M. P., L. B. Reller, J. R. Murphy, and K. A. Lichtenstein.** 1983. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev. Infect. Dis. **5**:35–53.
65. **Wenzel, R. P., C. A. Osterman, K. J. Hunting, and J. M. Gwaltney, Jr.** 1976. Hospital-acquired infections. I. Surveillance in a university hospital. Am. J. Epidemiol. **103**:251–260.
66. **Whiteside, M., J. Moore, and K. Ratzan.** 1983. An investigation of enterococcal bacteremia. Am. J. Infect. Control. **11**:125–129.
67. **Wilson, W. R., C. J. Wilkowske, A. J. Wright, M. A. Sande, and J. E. Geraci.** 1984. Treatment of streptomycin-susceptible and streptomycin-resistant enterococcal endocarditis. Ann. Intern. Med. **100**:816–823.
68. **Zervos, M. J., S. Dembinski, T. Mikesell, and D. R. Schaberg.** 1986. High-level resistance to gentamicin in *Streptococcus faecalis*: risk factors and evidence for exogenous acquisition of infection. J. Infect. Dis. **153**:1075–1083.
69. **Zervos, M. J., C. A. Kauffman, P. M. Therasse, A. G. Bergman, T. S. Mikesell, and D. R. Schaberg.** 1987. Nosocomial infection by gentamicin-resistant *Streptococcus faecalis*: an epidemiologic study. Ann. Intern. Med. **106**:687–691.
70. **Zervos, M. J., T. S. Mikesell, and D. R. Schaberg.** 1986. Heterogeneity of plasmids determining high-level resistance to gentamicin in clinical isolates of *Streptococcus faecalis*. Antimicrob. Agents Chemother. **30**:78–81.
71. **Zervos, M. J., J. E. Patterson, S. Edberg, C. Pierson, C. A. Kauffman, T. S. Mikesell, and D. R. Schaberg.** 1987. Single-concentration broth microdilution test for detection of high-level aminoglycoside resistance in enterococci. J. Clin. Microbiol. **25**:2443–2444.
72. **Zervos, M. J., M. S. Terpenning, D. R. Schaberg, P. M. Therasse, S. V. Medendorp, and C. A. Kauffman.** 1987. High-level aminoglycoside-resistant enterococci: colonization of nursing home and acute care hospital patients. Arch. Intern. Med. **147**:1591–1594.